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ORIGINAL ARTICLE

Gene-panel testing of breast and ovarian cancer patients identifies a recurrent *RAD51C* duplication

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Gene-panel sequencing allows comprehensive analysis of multiple genes simultaneously and is now routinely used in clinical mutation testing of high-risk breast and ovarian cancer patients. However, only *BRCA1* and *BRCA2* are often analyzed also for large genomic changes. Here, we have analyzed 10 clinically relevant susceptibility genes in 95 breast or ovarian cancer patients with gene-panel sequencing including also copy number variants (CNV) analysis for genomic changes. We identified 12 different pathogenic *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *CHEK2*, or *RAD51C* mutations in 18 of 95 patients (19%). *BRCA1/2* mutations were observed in 8 patients (8.4%) and *CHEK2* protein-truncating mutations in 7 patients (7.4%). In addition, we identified a novel duplication encompassing most of the *RAD51C* gene. We further genotyped the duplication in breast or ovarian cancer families (n = 1149), in unselected breast (n = 1729) and ovarian cancer cohorts (n = 553), and in population controls (n = 1273). Seven additional duplication carries were observed among cases but none among controls. The duplication associated with ovarian cancer risk (3/590 of all ovarian cancer patients, 0.5%, P = .032 compared with controls) and was found to represent a large fraction of all identified *RAD51C* mutations in the Finnish population. Our data emphasizes the importance of comprehensive mutation analysis including CNV detection in all the relevant genes.

KEYWORDS

breast cancer, gene-panel, ovarian cancer, RAD51C

1 | INTRODUCTION

Most of the familial risk of breast and ovarian cancer is still unexplained. The majority of the known predisposing mutations are in genes that function in DNA damage response and DNA repair. The most important breast and ovarian cancer susceptibility genes are *BRCA1* (OMIM *113705) and *BRCA2* (OMIM *600185) conferring on average 60% and 55% cumulative risks of breast cancer by the age of 70 and 59% and 16.5% risk of ovarian cancer, respectively.[1] Mutations in the other high-risk genes *TP53* (OMIM *191170), *PTEN* (OMIM *601728), *STK11* (OMIM *602216), and *CDH1* (OMIM *192090) confer an increased risk of breast cancer in the context of rare hereditary cancer-predisposition syndromes.[2–6] Mutations in *CHEK2* (OMIM *604373) and *PALB2* (OMIM *610355) confer a more moderate risk of breast cancer while mutations in *RAD51C* (OMIM *602774) and *RAD51D* (OMIM *602954) increase the risk of ovarian cancer.[7–10] In addition, a large number of common low-risk breast and ovarian cancer variants have been identified.[11,12] In the Finnish population, the majority of the mutations that have been observed in *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, and *RAD51D* are recurrent founder mutations.[13–17]

In the past, clinical genetic testing of high-risk individuals, for example familial or early-onset breast cancer cases, was mainly limited to the *BRCA1* and *BRCA2* genes. Since the development of next-generation sequencing techniques, multigene panel testing has been increasingly utilized. Several studies have reported approximately 4% to 6% frequency of mutations in genes other than *BRCA1/2* among hereditary breast and ovarian cancer patients.[18] If only *BRCA1/2* testing was performed, a large number of patients with potentially actionable mutations would not receive a molecular diagnosis. Furthermore, single-gene tests are not comprehensive as more than 1 risk variants may be segregating in some cancer families and thus a negative test result for a single gene would not be enough for risk stratification. The downside is that the commercially available gene-panels include a vast range of known and putative breast cancer susceptibility genes of which only some have clinical utility.[19] Some panels also include a broad range of cancer predisposition genes that may increase the risk of other malignancies but not breast cancer. Easton et al estimated that currently only *BRCA1*, *BRCA2*, *TP53*, *CDH1*, *PTEN*, *STK11*, *NF1*, *PALB2*, *CHEK2*, *ATM*, and *NBN* have sufficient evidence of association with breast cancer to have clinical validity and utility.[19] In addition to these genes, there is clear evidence of association with ovarian cancer for mutations in *RAD51C*, *RAD51D*, and *BRIP1* but the association with breast cancer is uncertain.[9,10,19,20]

Here, we have analyzed the *BRCA1*, *BRCA2*, *CDH1*, *PTEN*, *STK11*, *TP53*, *CHEK2*, *PALB2*, *RAD51C*, and *RAD51D* genes in 95 high-risk breast or ovarian cancer patients by gene-panel sequencing. We identified a novel *RAD51C* germline duplication which was further characterized for breast and ovarian cancer risk by screening the duplication in Finnish familial and unselected breast and ovarian cancer patients. We also performed expression analysis for *RAD51C* duplication carriers and non-carriers.

2 | MATERIALS AND METHODS

2.1 | Subjects

Gene-panel sequencing was performed for germline DNA samples of 95 unrelated breast or ovarian cancer patients ascertained at the Helsinki University Hospital Department of Clinical Genetics. The patients referred to genetic testing fulfilled the following criteria: at least 3 breast or ovarian cancer cases among first- or second-degree relatives including the proband (n = 35), 2 breast or ovarian cancer cases among first-degree relatives including the proband (n = 23), male breast cancer (n = 5), early-onset breast cancer cases diagnosed with breast cancer at the age of 40 or younger (n = 18), triple-negative breast cancer cases (TNBC, ie, estrogen receptor [ER], progesterone receptor [PR] and HER2 negative) diagnosed at the age of 50 or younger (n = 10), patients diagnosed with both breast and ovarian cancer with a family history of other cancers (n = 3), and a patient diagnosed with breast and colorectal cancer with family history of other cancers (n = 1). Information on family history of cancer was collected from patient interviews thereafter most of the cancer cases were verified from clinical records. Male relatives were excluded when calculating the degree of relationship for the breast cancer families. *BRCA1/2* screening had been previously performed for 17 patients with 13 patients screened negative for the Finnish founder mutations and 4 patients for the full genes. In addition, 2 patients had a family member previously screened negative for the full *BRCA1/2* genes and 1 patient had a family member previously screened negative for the *BRCA1/2* founder mutations, these family members were not tested with the gene-panel.

The identified *RAD51C* duplication was further genotyped in 1149 *BRCA1/2*-negative familial breast or ovarian cancer patients (including 1122 breast cancer patients, 19 patients with both breast and ovarian cancer, and 8 ovarian cancer patients), in 1729 unselected breast cancer patients, and in 553 unselected ovarian cancer patients ascertained at the Helsinki University Hospital as well as in 1273 healthy female population controls. Of the familial patients, 381 are included also in the unselected breast cancer cohort, 3 in the unselected ovarian cancer cohort, and 2 in both the unselected breast and unselected ovarian cancer cohorts. The patient cohorts are described in more detail in the Supporting Information, Appendix S1. The samples from the unselected breast cancer patients and breast and ovarian cancer families were germline DNA isolated from peripheral blood whereas 429 samples from the unselected ovarian cancer patients were genomic DNA and 124 were tumor DNA. Informed consent was obtained from all individual participants and the study was approved by the Ethics committee of the Helsinki University Hospital.

2.2 | Gene-panel sequencing

Ten genes (*BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PTEN*, *STK11*, *TP53*, *PALB2*, *RAD51C*, and *RAD51D*) included in a gene-panel, were analyzed in this study. The SureSelectXT Custom 3-5.9 Mb library kit (Agilent Technology, Santa Clara, CA, USA) was used for DNA capture and the sequencing was performed on the Illumina HiSeq 2500 (Illumina, San Diego, CA, USA; Appendix S1). Identified variants were annotated for their effect on protein coding transcripts and only protein-truncating mutations (ie, nonsense, splicing and frameshift indel mutations) and pathogenic missenses in the 10 studied genes were considered in this study. Copy number variants (CNVs) were identified using an in-house method based on the number of read pairs in short windows over the target regions as described in the Appendix S1. In addition, *BRCA1* and *BRCA2* large genomic changes were investigated by multiplex ligation-dependent probe amplification (MLPA). Pathogenic mutations in the studied genes were verified with Sanger sequencing.

2.3 | Duplication characterization

In the CNV analysis, a novel duplication covering the *RAD51C* exons 1 to 7 was identified. The exact size and location of the duplication was characterized with polymerase chain reaction (PCR) in a germline DNA sample of the carrier patient. The DNA was amplified using a forward primer 5′-CTGATCGTGCAGTTTGGGTC-3′ that binds to *RAD51C* intron 7 upstream of the duplication breakpoint and a reverse primer 5′-TTTTCTCGGCACCAAACCTT-3′ that binds to the duplicated sequence at the intergenic region upstream of *RAD51C*. The PCR products were Sanger sequenced as described in Appendix S1.

2.4 | Genotyping

Relatives of the index patient with available DNA samples and the unselected and familial breast and ovarian cancer cohorts and population controls were screened for the duplication with PCR assay. DNA samples were amplified using the same primer pair as in duplication characterization. A second reverse primer 5′-AGGGGGAGGATTTACCAGTC-3′ that binds to *RAD51C* intron 7 in the non-duplicated region was used as a control to monitor the success of the PCR reaction. The PCR products were run on a 2% agarose gel. Samples containing the duplication displayed 2 bands on the gel and samples without the duplication 1 band (Figure S1). All carriers were confirmed with a second PCR.

2.5 | Gene-expression analysis

Lymphoblastoid cell lines of 4 female breast cancer patients from the index family, including 3 duplication carriers and 1 non-carrier, as well as of 1 healthy, unrelated non-carrier woman were used for gene-expression analysis. Gene-expression analysis was performed with Cells-to-CT 1-Step TaqMan Kit (Ambion, Waltham, MA, USA) and TaqMan gene-expression assays (Applied Biosystems, Foster City, CA, USA) according to manufacturers' protocols (Appendix S1). Two *RAD51C* TaqMan gene-expression assays that bind to exons 1 to 2 and 8 to 9 were used to measure *RAD51C* expression and an *ACTB* assay was used as an endogenous control. The reference sample was the unrelated non-carrier woman. The relative expression was quantified using the comparative C_T method. Four independent experiments were performed with 3 technical PCR replicates.

Total RNA extracted with RNeasy Plus Mini kit (Qiagen, Hilden, Germany) from 3 carrier and 2 control wild type lymphoblastoid cell lines were subjected to reverse-transcription PCR with random primers using the SuperScript III RT-PCR kit (Invitrogen, Waltham, MA, USA). Complementary DNA (cDNA) template was used for PCR. PCR was set up using 2 controls and 3 carrier lymphoblastoid cell lines cDNA, no template control, and human genomic DNA control (to confirm that PCR product is not from genomic DNA). The templates were amplified using primers 5′-CTACACAGAGTTACTGGCAC-3′ and 5′-GTCCCAATGAAAGATTAGCCG-3′ in *RAD51C* exons 4 and 7 or primers 5′-CAGAACACTCAAAGGTTCGAC-3′ and 5′-AGAGAAACCATCGTATGTTCC-3′ in exons 4 to 5 junction and intron 7. The PCR products were analyzed on agarose gel.

2.6 | Statistical analyses

Statistical analyses were performed by using the R version 3.0.2 (http://www.r-project.org/). To evaluate the difference in the frequency of the duplication between cases and controls, 2-sided *P*-values were calculated using Fisher's exact test. The difference in the *RAD51C* expression was evaluated with Student's *t* test. A *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Mutations identified in the gene-panel testing

Out of the 95 patients tested with the gene-panel sequencing, 18 carried clearly pathogenic mutations in the genes included in this study (19%) (Tables 1 and 2). Altogether 12 different mutations were identified in these 18 patients (Table 1). Protein-truncating *BRCA1* mutations, including a large genomic change exon 13 duplication, were detected in 5 patients (5.3%) and *BRCA2* mutations in 3 patients (3.2%). The *CHEK2* c.1100delC mutation was detected in 7 patients (7.4%), 2 of which also carried the *CHEK2* splicing mutation c.444+1G>A. Further analysis of family members showed that the 2 *CHEK2* mutations were inherited in different alleles in both of these patients. One pathogenic missense mutation was identified in both the *PTEN* and *TP53* genes. In *RAD51C*, the Finnish founder mutation c.837+1G>A was observed in 1 patient who was affected with ovarian and uterine cancer. No deleterious mutations in *STK11*, *PALB2*, or *RAD51D* were detected. In addition to these pathogenic mutations, the low-penetrance *CHEK2* c.470T>C p.Ile157Thr (I157T) missense variant was detected in 6 patients, 2 of which were also *BRCA1* carriers and 1 *CHEK2* c.1100delC carrier.

Out of the 7 *CHEK2* c.1100delC carriers, 6 were women diagnosed with breast cancer at an early age (range 22-39 years) and 1 was a male patient affected with breast and papillary thyroid cancers (Table 1). The *PTEN* mutation carrier was diagnosed with breast cancer at the age of 39 and her mother and maternal aunt at the age of 63 and 45. The maternal aunt was also diagnosed with synovial sarcoma at the age of 43. After the result of the genetic test, the patient was inspected more carefully. Her head circumference was normal but she had a fibroma, goiter and several trichilemmomas. The *TP53* mutation carrier was diagnosed with bifocal breast cancer at the age of 29. Her maternal grandfather's sister had breast cancer at an old age and maternal aunt colon cancer at the age of 55. The *PTEN* and *TP53* mutations have been reported as pathogenic mutations in the ClinVar (RCV000169787.2 and RCV000129010.2) and HGMD databases and neither is observed in the ExAC database.

Pathogenic mutations were identified in 6 (17%) of the 35 patients from families with 3 or more affected members and in 5 (22%) of the 23 patients from families with 2 affected members (Table 2). Of the early-onset breast cancer patients, 4 (22%) were mutation positive whereas 20% of TNBC and male breast cancer patients harbored mutations. Of the 20 patients from families previously tested negative for *BRCA1/2* mutations, 1 harbored the *CHEK2* c.1100delC and c.444+1G>A mutations and 1 had a *BRCA1* nonsense mutation which was not previously detected as only the *BRCA1/2* founder mutations had been screened.

3.2 | Duplication of exons 1 to 7 in *RAD51C*

In addition to these 12 mutations detected in 18 patients, a novel large heterozygous duplication encompassing the *RAD51C* exons 1 to 7 was observed in 1 patient. She was diagnosed with breast cancer at the age of 40 and with basal cell carcinoma at the age of 45 and had been previously tested negative for *BRCA1/2* mutations. She also had 3 sisters affected with breast cancer. After Sanger sequencing, the mutation was characterized as a 64 179 base pairs (bp) long duplication starting 31 512 bp upstream of the *RAD51C* start codon and extending to the *RAD51C* intron 7 with a 10 bp insertion CTTTTGTGAG between the copies (c.-31512_965+1210dup{insCTTTTGTGAG} according to the extended HGVS nomenclature[21]) (Figure 1). In effect, there is an extra copy of the upstream sequence

TABLE 1 Pathogenic mutations observed in the 95 patients undergoing gene-panel testing

Mutation[a]	Cancer (dg-age)	Criterion	Histology[b]	Grade	ER	PR	HER2
***BRCA1* c.3626delT**	BC (34)	BC family ≥3 affected	Ductal	NA	neg	NA	NA
***BRCA1* c.3626delT**[c]	BC bilat (43 + 48)	TNBC	Ductal	3	neg	neg	neg
BRCA1 c.4656C>A p.Tyr1552Ter[c]	BC (52) and OC (58)	BC + OC family 2 affected	Ductal	1	pos	pos	NA
BRCA1 c.5278-1G>C	BC (31)	TNBC	Ductal	NA	neg	neg	neg
***BRCA1* c.4186-1787_4357 +4122dup** (ex13 duplication)	BC bilat (54 + 54)	BC + OC family ≥3 affected	Ductal	3	neg	neg	neg
***BRCA2* c.1286T>G p.Leu429Ter**	BC (38)	Early-onset BC	Ductal	3	neg	neg	pos
***BRCA2* c.7480C>T p.Arg2494Ter**	BC (60) and OC (54)	BC + OC family ≥3 affected	Ductal	NA	pos	neg	neg
BRCA2 c.8314G>T p.Glu2772Ter	BC (26)	BC family 2 affected	Ductal	2-3	pos	pos	pos
***CHEK2* c.1100delC**[c]	BC (22) bifocal	BC family 2 affected	Ductal	3	pos	pos	pos
***CHEK2* c.1100delC**	BC (34)	BC family 2 affected	Ductal	3	pos	neg	neg
***CHEK2* c.1100delC**	BC (25)	Early-onset BC	Ductal	2	pos	pos	pos
***CHEK2* c.1100delC**	BC (26)	Early-onset BC	Ductal	2	pos	pos	pos
***CHEK2* c.1100delC**	BC (30)	BC family ≥3 affected	Ductal	3	pos	neg	pos
***CHEK2* c.1100delC**; ***CHEK2* c.444+1G>A**	BC bilat (39 + 69)	BC family ≥3 affected	Ductal	2	NA	NA	NA
***CHEK2* c.1100delC**; ***CHEK2* c.444+1G>A**	BC (46) and thyroid (28)	MBC	Ductal	2	pos	pos	neg
PTEN c.70G>T p.Asp24Tyr	BC (39)	BC family ≥3 affected	Duct et lobular	3	pos	pos	neg
***RAD51C* c.837+1G>A**	OC (40) and uterine (40)	BC + OC family 2 affected	Serous	3	pos	NA	NA
TP53 c.844C>G p.Arg282Gly	BC (29) bifocal	Early-onset BC	Ductal	NA	pos/neg	pos/neg	pos/pos

Abbreviations: BC, breast cancer; bilat, bilateral; dg-age, age at diagnosis; ER, estrogen receptor; MBC, male breast cancer; NA, not available; neg, negative; OC, ovarian cancer; pos, positive; PR, progesterone receptor; TNBC, triple-negative breast cancer.

Recurrent or founder mutations in the Finnish population are highlighted in bold.

[a] The coding refers to *BRCA1* transcript NM_007294.3 (U14680.1 exon numbers), *BRCA2* transcript NM_000059.3, *CHEK2* transcript NM_007194.3, *PTEN* transcript NM_000314.6, *RAD51C* transcript NM_058216.2 and *TP53* transcript NM_000546.5.

[b] Tumor histology, grade, and ER, PR, and HER2 status is denoted for the first breast tumor except for the patient without breast cancer the information is given for the ovarian tumor.

[c] Patient also carries the *CHEK2* c.470T>C p.Ile157Thr variant.

TABLE 2 Number of mutation carriers in each patient group

Ascertainment criteria	Total	Mutation carriers (%)	Mutated genes (number of families with mutations in the gene)
BC family ≥3 affected	35	6 (17%)	*BRCA1* (2), *BRCA2* (1), *CHEK2* (2), *PTEN* (1)
BC only (≥3 affected)	20	4 (20%)	*BRCA1* (1), *CHEK2* (2), *PTEN* (1)
BC + OC (≥3 affected)	15	2 (13%)	*BRCA1* (1), *BRCA2* (1)
BC family 2 affected	23	5 (22%)	*BRCA1* (1), *BRCA2* (1), *CHEK2* (2), *RAD51C* (1)
BC only (2 affected)	14	3 (21%)	*BRCA2* (1), *CHEK2* (2)
BC + OC (2 affected)	9	2 (22%)	*BRCA1* (1), *RAD51C* (1)
Early-onset BC	18	4 (22%)	*BRCA2* (1), *CHEK2* (2), *TP53* (1)
TNBC	10	2 (20%)	*BRCA1* (2)
MBC	5	1 (20%)	*CHEK2* (1)
other[a]	4	0 (0%)	—
All	95	18 (19%)	

Abbreviations: BC, breast cancer; MBC, male breast cancer; OC, ovarian cancer; TNBC, triple-negative breast cancer.

[a] includes 3 BC and OC patients and 1 BC and colorectal cancer patient with a family history of other cancers.

and the *RAD51C* exons 1 to 7 located 31 kb upstream of the normal copy of the *RAD51C* gene.

We screened the duplication with a PCR assay in the relatives of the duplication carrier with available DNA samples (Figure S1). Two of the index patient's 3 sisters affected with breast cancer were carriers whereas no duplications were observed in the 3 tested healthy women. Other cancer cases in the family included ovarian cancer and various other cancer types such as Hodgkin lymphoma, colorectal cancer, and prostate cancer among distant relatives, but no samples were available from these patients. We further genotyped the duplication in familial breast and ovarian cancer patients (n = 1149), in unselected cohorts of breast (n = 1729) and ovarian (n = 553) cancer

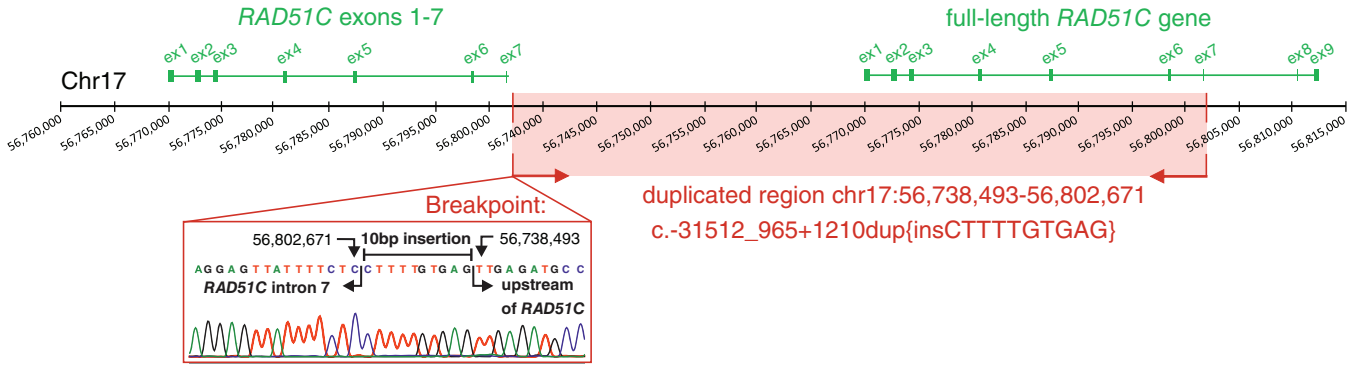

FIGURE 1 Diagram of the *RAD51C* duplication which starts 31 kb upstream of the *RAD51C* gene and covers exons 1 to 7. The duplication is highlighted in light red. Exon numbering is based on *RAD51C* transcript NM_058216.2 and the chromosome positions are according to build hg19 (GRCh37)

cases, and in healthy female population controls (n = 1273) collected from the same geographic region. Seven additional duplication carriers were identified among cases and none among controls. Two of them were familial breast cancer patients, 3 were unselected breast cancer patients with 1 of them also affected with ovarian cancer, and 2 were unselected ovarian cancer patients. As *RAD51C* mutations have been previously associated with ovarian cancer,[9,16] we performed subgroup analyses based on family history and personal history of breast and ovarian cancer in order to define the patient group with the strongest association (Table 3). Altogether, half of the 8 duplication carriers were affected with ovarian cancer or had a family history of ovarian cancer. The duplication was more frequent among ovarian cancer cases (0.5%, P = .032) as well as among patients with a personal or family history of ovarian cancer (0.5%, P = .021) than among population controls (0.0%) (Table 3). The frequency of the duplication among breast cancer patients was 0.2% with a slightly higher 0.3% frequency among familial patients but did not significantly differ from controls (P > .05). All the duplication carriers were negative for the Finnish *RAD51C* founder mutations c.93delG and c.837+1G>A.

To investigate if the duplication is transcribed into mRNA and whether it affects *RAD51C* expression we quantified the *RAD51C* RNA levels in lymphoblastoid cell lines of 3 carriers and 2 non-carriers. We first measured the *RAD51C* expression with a probe binding to *RAD51C* exons 1 to 2 boundary and used *ACTB* as an endogenous control. Two of the duplication carriers showed significant, 1.52- to 1.68-fold, increased *RAD51C* expression compared with the non-carrier control sample (P = .020 and P = .013) with the third carrier showing 1.23-fold increase (P = .141) (Figure 2 and Table S1). On average, the 3 carriers had 1.48-fold increased *RAD51C* expression (P = .074 compared with the 2 non-carries). When the expression was measured using a probe binding to exons 8 to 9 boundary, residing outside of the duplicated area, the expression levels of the carriers and non-carriers were comparable. When the *RAD51C* exons 8 to 9 probe was used as a control and the expression was measured with the *RAD51C* exon 1 to 2 probe, the duplication carriers showed 1.21- to 1.66-fold increased expression, 1 of them with significantly increased expression compared with the control (P = .006). On average, the 3 carriers had 1.42-fold expression of the *RAD51C* ex1-2 (P = .084 compared with the 2 non-carriers).

TABLE 3 Frequencies of the *RAD51C* duplication among breast and ovarian cancer patients and in different subgroups as defined by family history and personal history of cancer

Patient group	Total	Wt (%)	Dup (%)	*P*-value
Population controls	1273	1273 (100.0%)	0 (0.0%)	
All BC[a]	2533	2527 (99.8%)	6 (0.2%)	.188
Unselected BC cohort	1729	1726 (99.8%)	3 (0.2%)	.267
Familial BC[b] cohort	1187	1184 (99.7%)	3 (0.3%)	.112
BC only[c]	973	971 (99.8%)	2 (0.2%)	.188
BC + OC[d]	214	213 (99.5%)	1 (0.5%)	.144
All OC[e]	590	587 (99.5%)	3 (0.5%)	**.032**
Unselected OC cohort	553	551 (99.6%)	2 (0.4%)	.092
Familial OC[f]	8	8 (100.0%)	0 (0.0%)	1
Any OC[g]	782	778 (99.5%)	4 (0.5%)	**.021**

Abbreviations: BC, breast cancer; OC, ovarian cancer; dup, duplication carrier; wt, wild type.

The patient groups are overlapping and described in more detail in Appendix S1.

[a] All BC patients from the unselected and familial BC cohorts, 36 (including 1 duplication carrier) are BC + OC patients included also in All OC.

[b] *BRCA1/2*-negative familial BC and BC + OC patients from the gene-panel sequencing (n = 46) were combined with the genotyped patients including also the patient where the duplication was discovered.

[c] BC families with no known family history of OC.

[d] BC families with at least 1 OC case among family members or distant relatives.

[e] All OC includes unselected and familial OC patients and BC + OC patients from the unselected and familial BC cohorts.

[f] OC patients with a family history of OC but not BC.

[g] Any OC includes all patients with personal or family history of OC.

To further characterize the mRNA produced from the duplication, total RNA was extracted from lymphoblastoid cell lines of 3 duplication carriers and 2 wild type controls. If the translation continues into *RAD51C* intron 7, a new stop codon is predicted to appear after 18 amino acids p.(Phe323SerfsTer18). Using a forward primer in exon 4 and a reverse primer in exon 7, an expected 300 bp long product was observed in both the controls and carriers (Figure S2). When using a forward primer at the exon 4 to 5 junction and a reverse primer in intron 7, a novel 663 bp long product was observed in the carriers but not in the controls.

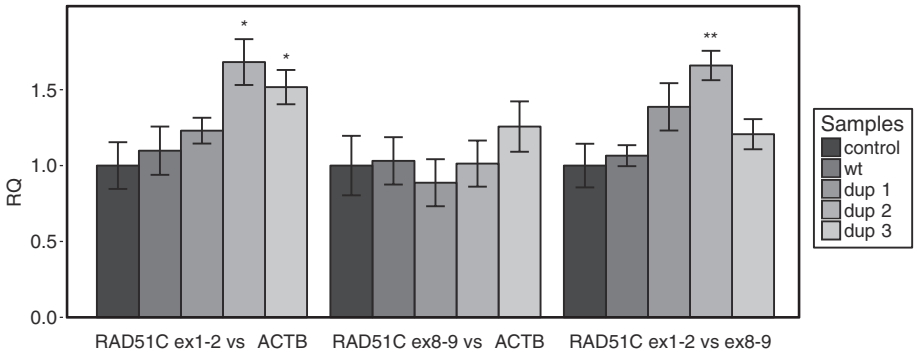

FIGURE 2 *RAD51C* expression in 3 duplication carriers (dup 1-3) and 1 non-carrier (wt) normalized to a healthy non-carrier (control). Bars represent means of 4 independent experiments ± SEM. *P*-values for each sample compared with the control are calculated with Student's *t* test, **P* < 0.05, ***P* < 0.01. RQ, relative quantification; dup, duplication carrier; wt, wild type

4 | DISCUSSION

The multigene panel testing of 95 high-risk breast or ovarian cancer patients revealed pathogenic mutations in 18 patients (19%) and a novel *RAD51C* ex1-7 duplication in 1 patient. While mutations in the most important susceptibility genes *BRCA1* and *BRCA2* were observed in 5.3% and 3.2% of the patients, respectively, the highest mutation frequency of 7.4% was in *CHEK2*.

4.1 | Mutations in different patient groups and characteristics of the mutation carriers

The only mutation observed in an ovarian cancer case not affected with breast cancer was the *RAD51C* c.837+1G>A mutation while in patients affected with both breast and ovarian cancer *BRCA1* and *BRCA2* mutations were observed. This is consistent with an increased risk of ovarian cancer but not breast cancer for *RAD51C* mutation carriers and an increased risk of both breast and ovarian cancer for *BRCA1/2* mutation carriers.[1,9,16] The 2 mutations detected among the 10 patients ascertained because of TNBC were in *BRCA1*, consistent with *BRCA1* tumors being often triple-negative.[22] *CHEK2* mutations were detected in women diagnosed with breast cancer at an early age and in a man diagnosed with breast and thyroid cancers. This is in line with previous studies indicating that the relative breast cancer risk of *CHEK2* c.1100delC carriers decreases by age and showing an association between *CHEK2* mutations and the risk of male breast cancer and thyroid cancer.[23–26]

Single *PTEN* and *TP53* mutations were observed in patients who did not present the most typical features of the PTEN hamartoma tumor syndrome (PHTS) or Li-Fraumeni syndrome (LFS) caused by germline mutation in these genes, respectively. PHTS is characterized by macrocephaly, multiple hamartomas, and increased risks of malignant and benign tumors, mainly of the breast, thyroid, and endometrium and LFS by a high risk and an early onset of cancer, most commonly breast cancer, childhood sarcomas, brain tumors, and adrenocortical carcinoma.[2–4] Interestingly, the identified *PTEN* mutation carrier had features of Li-Fraumeni-like syndrome with an early-onset breast cancer and a family history of breast cancer and sarcoma. After the genetic test result, however, she was found to have a fibroma, goiter and trichilemmomas typical for PHTS. The *TP53* mutation carrier had an early-onset breast cancer and a family history of breast and colon cancer but did not fulfill the Chompret criteria for *TP53* testing.[3] The detected *PTEN* and *TP53* missense mutations have been previously reported in PHTS and LFS families and the *TP53* c.844C>G p.Asp24Tyr has been shown to function as a dominant negative allele.[27–29]

4.2 | Double heterozygotes

Two patients carried 2 different protein-truncating mutations (2.1% of the tested patients, 8.7% of the mutation carriers). In previous gene-panel studies, Walsh et al observed 2 different mutations in 3 out of 360 unselected ovarian cancer patients (0.8% of tested, 3.7% of mutation carriers) and Tung et al in 5 out of 1781 breast cancer patients (0.3% of tested, 2.1% of mutation carriers).[30,31] Carriers of 2 mutations may have a higher risk than carriers of a single mutation and low-penetrance variants may modify the risk of other mutations. Both observed double heterozygotes in our study carried the *CHEK2* mutations c.1100delC and c.444+1G>A. In addition, 3 patients with a truncating mutation in *BRCA1* or *CHEK2* carried the low-penetrance *CHEK2* missense c.470T>C. *CHEK2* compound heterozygotes have been estimated to have a higher risk of breast cancer than women with a single *CHEK2* mutation whereas a *CHEK2* mutation in a *BRCA1* positive woman may not increase the risk beyond that of the *BRCA1* mutation alone.[32] Moreover, 2 of the patients previously tested negative for *BRCA1/2* mutations were now found to harbor either *CHEK2* or *BRCA1* mutations and a third patient carried the *RAD51C* ex1-7 duplication. Lincoln et al compared traditional testing with multigene panel testing and observed deleterious mutations in other breast cancer genes in 2 patients previously tested negative for the familial *BRCA1/2* mutation and in 2 *BRCA1/2* positive patients.[33] Our results, together with other studies, highlight the advantage of gene-panel testing as information for multiple genes is gained simultaneously.

4.3 | *RAD51C* duplication

Two genomic changes were detected among the patients: the known pathogenic *BRCA1* exon 13 duplication and a novel 64 kb long duplication encompassing most of the *RAD51C* gene. The observed 0.5% frequency of the *RAD51C* duplication among ovarian cancer patients and its absence among population controls ($P = .032$) suggest an increased risk of ovarian cancer, consistent with previous studies on *RAD51C*.[9,16] The cumulative risk of ovarian cancer by age 80 for *RAD51C* mutation carriers has been estimated to be >9% whereas there was no evidence for increased breast cancer risk (relative risk [RR] = 0.91, $P = .8$).[9] We have previously identified 2 recurrent

deleterious *RAD51C* point mutations, c.93delG and c.837+1G>A, that were each observed at a 0.5% frequency among unselected ovarian cancer patients.[16] This is comparable to the 0.4% duplication frequency among unselected ovarian cancer patients. The *RAD51C* point mutations increased the risk of ovarian cancer (odds ratio [OR] = 6.31, 95% confidence interval [CI]1.15-34.6, P = .033 for unselected ovarian cancer cases compared with population controls) but did not associate with breast cancer susceptibility in the absence of ovarian cancer family history. It appears likely, that in *RAD51C* carrier families with breast and ovarian cancer, there may be also other alleles conferring increased breast cancer risk.

When combining the duplication and the point mutations, we have observed 17 *RAD51C* mutation carriers among Finnish breast or ovarian cancer patients with a combined frequency of 1.4% among unselected ovarian cancer patients and 2.1% among patients with personal or family history of ovarian cancer. The frequency is markedly higher than a 0.32% *RAD51C* overall mutation frequency among unselected ovarian cancer patients observed in a recent large population-based study.[34] This highlights the significance of recurrent mutations in Finland.

In the ExAC database, a duplication encompassing the *RAD51C* exons 1 to 7 has been observed in 12 of 3301 Finnish samples representing diverse population cohorts from different parts of the country. This CNV may represent the same duplication as observed here. There are limitations of use of ExAC CNV data as variant calls and frequency estimates are generated from targeted exome sequencing with short reads, and rarity of events may compromise accurate frequency estimates.[35] However, due to the population history of Finland, the allele frequencies differ between different geographical regions and there are genetically distinct subisolates.[36] Thus, geographical matching of cases and controls is important. In our study, the population controls and the cases were collected from the same geographical area. The ExAC CNV carriers may originate from a different region of Finland suggesting also that the detected *RAD51C* CNV may be more prevalent in another part of the country. Further validation of the association in larger datasets is warranted.

The identified duplication covers the first 7 exons of *RAD51C* which are translated into amino acids 1 to 322 in the wild type protein. Assuming the translation continues into the intron 7, a new stop codon is predicted after 18 amino acids p.(Phe323SerfsTer18). Consistent with this, a novel mRNA isoform was observed in the duplication carriers. The heterozygous carriers have 3 copies of the *RAD51C* exons 1 to 7 and 2 copies of the exons 8 to 9 in their genomes. This is in line with the approximately 1.4-fold expression of the *RAD51C* exons 1 to 2 compared with the exons 8 to 9 in the duplication carriers although the increase in expression compared with the non-carriers did not reach statistical significance due to the small sample size (P = .084). The results of the expression analysis suggest that a stable aberrant mRNA may be produced. If the duplication is translated into a truncated protein, it might disturb the complex formation of RAD51C. The RAD51C protein functions at DNA double-strand break repair via homologous recombination and forms 2 distinct complexes with the other RAD51 paralogs: the BCDX2 complex with RAD51B, RAD51D, and XRCC2 and the CX3 complex with XRCC3.[37,38] The C-terminal amino acids 79 to 376 of RAD51C are needed for the binding to RAD51B, RAD51D, or XRCC3 and a complete β-sheet in the C-terminus is required for a proper folding of the protein.[39] Almost the entire RAD51C region, except for a few N-terminal residues, may be needed for the XRCC3 binding.[40] Thus, the putative truncated RAD51C protein may not be able to bind the other paralogs as it lacks the C-terminal amino acids that are critical to binding. Besides the RAD51 paralogs, RAD51C also directly binds to PALB2 and forms a complex with PALB2, BRCA2, and RAD51.[41] Further functional studies are warranted to characterize the effect of the duplication on the protein level and to establish the level of pathogenicity.

In conclusion, the gene-panel testing of 95 high-risk breast or ovarian cancer patients revealed 12 pathogenic *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *CHEK2*, or *RAD51C* mutations in 18 patients, with 2 patients carrying more than 1 truncating mutation. The *RAD51C* ex1-7 duplication was identified as novel, likely pathogenic, recurrent mutation in the Finnish population associating with the risk of ovarian cancer and representing a large fraction of all identified *RAD51C* mutation carriers. This data emphasizes the importance of comprehensive mutation analysis of all the relevant genes simultaneously and including a method to detect CNVs.

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Conflict of interest

None of the authors has any conflict of interest to disclose.

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REFERENCES

1. Mavaddat N, Peock S, Frost D, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *J Natl Cancer Inst*. 2013;105:812-822.
2. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*. 1990;250:1233-1238.
3. McBride KA, Ballinger ML, Killick E, et al. Li-Fraumeni syndrome: cancer risk assessment and clinical management. *Nat Rev Clin Oncol*. 2014;11:260-271.
4. Ngeow J, Sesock K, Eng C. Breast cancer risk and clinical implications for germline PTEN mutation carriers. *Breast Cancer Res Treat*. 2017;165:1-8.
5. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am J Gastroenterol*. 2010;105:1258-1264.

6. Hansford S, Kaurah P, Li-Chang H, et al. Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. *JAMA Oncol*. 2015;1:23-32.
7. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet*. 2002;31:55-59.
8. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med*. 2014;371:497-506.
9. Loveday C, Turnbull C, Ruark E, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. *Nat Genet*. 2012;44:475-476.
10. Loveday C, Turnbull C, Ramsay E, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. *Nat Genet*. 2011;43:879-882.
11. Michailidou K, Beesley J, Lindstrom S, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. *Nat Genet*. 2015;47:373-380.
12. Kuchenbaecker KB, Ramus SJ, Tyrer J, et al. Identification of six new susceptibility loci for invasive epithelial ovarian cancer. *Nat Genet*. 2015;47:164-171.
13. Sarantaus L, Huusko P, Eerola H, et al. Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. *Eur J Hum Genet*. 2000;8:757-763.
14. Vahteristo P, Bartkova J, Eerola H, et al. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. *Am J Hum Genet*. 2002;71:432-438.
15. Erkko H, Xia B, Nikkilä J, et al. A recurrent mutation in PALB2 in Finnish cancer families. *Nature*. 2007;446:316-319.
16. Pelttari LM, Heikkinen T, Thompson D, et al. RAD51C is a susceptibility gene for ovarian cancer. *Hum Mol Genet*. 2011;20:3278-3288.
17. Pelttari LM, Kiiski J, Nurminen R, et al. A Finnish founder mutation in RAD51D: analysis in breast, ovarian, prostate, and colorectal cancer. *J Med Genet*. 2012;49:429-432.
18. Graffeo R, Livraghi L, Pagani O, Goldhirsch A, Partridge AH, Garber JE. Time to incorporate germline multigene panel testing into breast and ovarian cancer patient care. *Breast Cancer Res Treat*. 2016;160:393-410.
19. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med*. 2015;372:2243-2257.
20. Ramus SJ, Song H, Dicks E, et al. Germline mutations in the BRIP1, BARD1, PALB2, and NBN genes in women with ovarian cancer. *J Natl Cancer Inst*. 2015. https://doi.org/10.1093/jnci/djv214.
21. Taschner PE, den Dunnen JT. Describing structural changes by extending HGVS sequence variation nomenclature. *Hum Mutat*. 2011;32:507-511.
22. Stevens KN, Vachon CM, Couch FJ. Genetic susceptibility to triple-negative breast cancer. *Cancer Res*. 2013;73:2025-2030.
23. Wasielewski M, den Bakker MA, van den Ouweland A, et al. CHEK2 1100delC and male breast cancer in the Netherlands. *Breast Cancer Res Treat*. 2009;116:397-400.
24. Pritzlaff M, Summerour P, McFarland R, et al. Male breast cancer in a multi-gene panel testing cohort: insights and unexpected results. *Breast Cancer Res Treat*. 2017;161:575-586.
25. Siolek M, Cybulski C, Gasior-Perczak D, et al. CHEK2 mutations and the risk of papillary thyroid cancer. *Int J Cancer*. 2015;137:548-552.
26. Schmidt MK, Hogervorst F, van Hien R, et al. Age- and tumor subtype-specific breast cancer risk estimates for CHEK2*1100delC carriers. *J Clin Oncol*. 2016;34:2750-2760.
27. Monti P, Perfumo C, Bisio A, et al. Dominant-negative features of mutant TP53 in germline carriers have limited impact on cancer outcomes. *Mol Cancer Res*. 2011;9:271-279.
28. Tok Celebi J, Chen FF, Zhang H, Ping XL, Tsou HC, Peacocke M. Identification of PTEN mutations in five families with Bannayan-Zonana syndrome. *Exp Dermatol*. 1999;8:134-139.
29. Poli T, Lagana F, Caradonna L, Gobbi R, Corradi D, Sesenna E. Primary orbital liposarcoma in Li-Fraumeni cancer family syndrome: a case report. *Tumori*. 2005;91:96-100.
30. Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci U S A*. 2011;108:18032-18037.
31. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer*. 2015;121:25-33.
32. Cybulski C, Gorski B, Huzarski T, et al. Effect of CHEK2 missense variant I157T on the risk of breast cancer in carriers of other CHEK2 or BRCA1 mutations. *J Med Genet*. 2009;46:132-135.
33. Lincoln SE, Kobayashi Y, Anderson MJ, et al. A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients. *J Mol Diagn*. 2015;17:533-544.
34. Song H, Dicks E, Ramus SJ, et al. Contribution of germline mutations in the RAD51B, RAD51C, and RAD51D genes to ovarian cancer in the population. *J Clin Oncol*. 2015;33:2901-2907.
35. Ruderfer DM, Hamamsy T, Lek M, et al. Patterns of genic intolerance of rare copy number variation in 59,898 human exomes. *Nat Genet*. 2016;48:1107-1111.
36. Jakkula E, Rehnström K, Varilo T, et al. The genome-wide patterns of variation expose significant substructure in a founder population. *Am J Hum Genet*. 2008;83:787-794.
37. Suwaki N, Klare K, Tarsounas M. RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. *Semin Cell Dev Biol*. 2011;22:898-905.
38. Masson JY, Tarsounas MC, Stasiak AZ, et al. Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev*. 2001;15:3296-3307.
39. Miller KA, Sawicka D, Barsky D, Albala JS. Domain mapping of the Rad51 paralog protein complexes. *Nucleic Acids Res*. 2004;32:169-178.
40. Kurumizaka H, Enomoto R, Nakada M, Eda K, Yokoyama S, Shibata T. Region and amino acid residues required for Rad51C binding in the human Xrcc3 protein. *Nucleic Acids Res*. 2003;31:4041-4050.
41. Park JY, Singh TR, Nassar N, et al. Breast cancer-associated missense mutants of the PALB2 WD40 domain, which directly binds RAD51C, RAD51 and BRCA2, disrupt DNA repair. *Oncogene*. 2014;33:4803-4812.

SUPPORTING INFORMATION

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